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Preliminary Observations on the Efficacy of Antidiabetic Plant, Tithonia Diversifolia (Hemsl.) A Gray (Tree Marigold) on Alloxan-Induced Diabetic Wistar Rats

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Abstract

Aim and Objectives: *The world-threatening increases of types 1 and 2 diabetes mellitus and their pressure on insulin and other drugs led to the continued search for non-conventional drugs as remedy particularly in countries with reduced economic growth. Therefore, the traditional use of Tithonia diversifolia leaves as an antidiabetic medicinal plant was investigated for its documentation into a data bank for further technological development and enhancement of its antidiabetic capacities to meet the challenges of diabetes mellitus on animal and human population.*

Design and Methods: *Eighteen (18) adult Wistar rats of both sexes, were placed at random into four groups and treated as follows: Group 1: Non-diabetic rats (ND) dosed orally with normal saline at 2 ml/kg.bd.wt. (n=3) served as normal control; Group 2: Diabetic rats that were left untreated (DU) (n=4) served as negative control; Group 3: Diabetic rats orally administered with crude ethanolic extracts of Anogeissus leicarpus stem bark as standard, (DEEAlSB) at a dose of 1,000 mg/kg.bd.wt. daily, (n=6), served as positive control; Group 4: Diabetic rats orally administered with crude ethanolic extracts of Tithonia diversifolia leaves (DEETdL) at a dose of 1,000 mg/kg.bd.wt. daily, (n=5).*

Administrations commenced immediately hyperglycaemia occurred in the alloxan-induced diabetic rats and when hyperglycaemia subsided in the diabetic rats, administrations were withdrawn. Random blood glucose (RBG) values were monitored post withdrawal of administrations.

Google search, National Institute of Health, Research Direct, Research Gate and Library search for peer review articles, were utilized for the study.

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Results: *Alkaloids, glycosides, phenolic compounds, tannins, steroids, carbohydrates, flavonoids, and terpenoids phytochemicals/phytconstituents are presented in the ethanolic extracts of T. diversifolia leaves, while saponins and anthraquinones are absent. Of these ten phytochemicals, only atraquinones were absent in the standard, crude ethanolic extracts of Anogeissus leiocarpus stem bark, applied in the current study.*

RBG values in normal control, ND (group 1) ranged between 92 and 129 mg/dl during the study; diabetic untreated rats, negative control. DU (group 2) and diabetic rats, orally administered the test sample, crude ethanolic extracts of T. diversifolia leaves, DEETdL, (group 4) developed an overwhelming hyperglycaemia one day post induction of diabetes, except one rat in the latter group 4, which developed hyperglycaemia three days post induction of diabetes. Group 3 diabetic rats assigned for treatment with the standard, crude ethanolic extracts of A. leiocarpus stem bark, DEEAlSB developed hyperglycaemia eight days post induction of diabetes, a delay that was ascribed to an observed enlarged body mass index (BMI), although one rat was overwhelmingly hypoglycaemic.

Diabetic untreated rats died from an overwhelming hyperglycaemia, by day 4 post induction of diabetes.

Oral administration of the standard, as from eight days post induction, revealed its effectiveness and efficacy in treating diabetes mellitus as from four days and completely effective on seven, nine and eleven days post treatment of diabetes, which led to the withdrawal of standard on eleven days post administration.

The diabetic rats administered the test sample, crude ethanolic extracts of T. diversifolia leaves, one day post induction of diabetes, responded very slowly, displaying some levels of effectiveness five, six twelve, fourteen and sixteen days post treatment with reversals in between, which led to its withdrawal eighteen days post administration.

Conclusion: *Post withdrawal of administration of standard revealed maintenance of normoglycaemia for sixteen days; while with the test sample, ethanolic extracts of T. diversifolia leaves, moderate hyperglycaemia existed for sixteen days post withdrawal, suggestive of continuous daily usage of T. diversifolia leaves as an antidiabetic remedy.*

Keywords: *Tithonia Diversifolia Leaf, Antidiabetic Potentials, Post Withdrawal, Mandatory Daily Usage.*

INTRODUCTION

The current projection that provided an ascendency of the more devastating type 2 diabetes mellitus (T2DM) and its complications, at 700.2 million adults by 2045 [1] requires an aggressive strategy towards the development of alternative non-conventional drug for the treatment of DM and its complications.

The successes achieved by Traditionalists in the management of DM with medicinal plants shifted attention towards research on traditionally identified medicinal plants with antidiabetic activities. Systematically programmed studies will harness relevant medicinal plants for technological advancement.

Tithonia diversifolia [2, 3, 4, 5, 6] of the family, *Asteraceae*, with common names (a). Tree Marigold, (b). Tithonia and (c) Mexican Sun Flower is widespread in many Nigerian ecological terrains and many other countries of Africa, Asia and South America. *T. diversifolia*'s agricultural usefulness was identified as the source of enhanced livestock feeds in silage preparation [7], a process that led to reduction of anti-nutrient phytoconstitutents, (phytin, tannin, oxalate, alkaloid and flavonoid) with length of ensiling [7].

Numerous medicinal benefits of *T. diversifolia* included antibacterial, antioxidant, treatment of malarial fever, anti-inflammatory, antidiabetes, treatment of hepatitis and pain [8].

T. diversifolia had long been in use, traditionally and successfully to control diabetes mellitus, simply by chewing the leaves periodically.

The objective of this study is to investigate on the qualitative phytochemical constituents, the antidiabetic activities of *T. diversifolia* and have a detailed documentation and/or data bank of antidiabetic medicinal plants with potential for technological advancements.

MATERIALS AND METHODS

Plant Collection, Extraction and Qualitative Phytochemical Screening:

Collection

The leaves of *Tithonia diversifolia* were harvested from Jaji village, along Zaria-Kaduna road, Kaduna State of Nigeria.

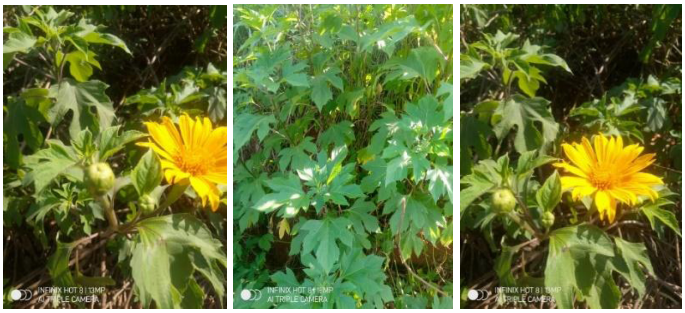

Fig.1. Tithonia diversifolia (HEMSL.) A GRAY (TREE MARIGOLD)

The leaves, harvested from fully and ubiquitously grown plants along the road-sides, were authenticated in the herbarium of the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria, with a sample voucher number of ABU0266. The leaves were dried for over four weeks, under shade at room temperature. Thereafter, pulverisation was performed with mortar and pestle.

Crude Ethanolic Extraction of Leaves of Tithonia diversifolia

Cold maceration method was utilized, with 95% v/v ethanol, to produce the crude ethanolic extract of the plant's leaves. One hundred (100) g of the powdered leaf material were poured into a separating funnel layered with packed cotton wool at its bottom. Complete immersion of the powdered material was accomplished for a 48 h. extraction, at room temperature, by using five hundred (500) ml of 95% v/v ethanol [9].

Qualitative Phytochemical Screening of Crude Ethanolic Extracts of Tithonia diversifolia Leaves

Detection of the different phytochemical constituents in the crude ethanolic extracts of *Tithonia diversifolia* leaves was achieved following standard procedures [10,11,12].

Determination of LD50 for Ethanolic Extract of T. diversifolia Leaves

Five Wistar rats were administered orally, with a limit dose of 5,000 mg/kg. bd. wt. and observed for a period of 24 hours and thereafter for 72 hours. There was no mortality or signs of toxicity, following the administration of the crude ethanolic extracts of *T. diversi*folia leaves.

Experimental Animals

Eighteen (18) adult Wistar rats of both sexes, were selected at random, from a group of forty-eight (48), weighing between 96 g and 143 g that were sourced from Department of Pharmacology and Toxicology, Ahmadu Bello University, Zaria. These eighteen (18) rats were placed at random, into four (4) groups, as follows:

Group 1: Non-Diabetic rats – ND; diabetes was not induced and each rat received normal saline orally, at a dose of 2 ml/kg. bd. wt. daily and served as normal control; (n=3)

Group 2: Diabetic Untreated rats – DU; diabetes was induced, left untreated and served as negative control; (n=4)

Group 3: Diabetic rats and ethanolic extract of *Anogeissus leiocarpus* stem bark treated – DEE*Al*SB; diabetes was induced and treated with ethanolic extract of *Anogeissus leiocarpus* stem bark, orally at a dose of 1000 mg/kg. bd. wt. daily and served as standard and positive control; (n=6)

(Shrub-like type) (Tree type)

Fig. 2. Anogeissus leiocarpus [Reference 21]

Group 4: Diabetic rats and ethanolic extract of *Tithonia diversifolia* leaf treated – DEE*Td*L; diabetes was induced and treated with ethanolic extract of *Tithonia diversifolia* leaf orally, at a dose of 1000 mg/kg. bd. wt. daily; (n=5)

Administration was performed using 18 G cannula oral gavage.

Induction of Diabetes Mellitus Using Alloxan Monohydrate

Diabetes mellitus was induced using alloxan monohydrate to produce type 1 diabetes mellitus in the Wistar rats of groups 2, 3, and 4 as described in an earlier study [12].

Alloxan monohydrate was reconstituted in cold normal saline to obtain a concentration of 200 mg/ml and administered rapidly intraperitoneally at a dose of 100 mg/kg. bd. wt. The rats were allowed an intake of 10% dextrose to prevent fatal hypoglycaemic shock along with continuous monitoring for fatal clinical signs. Rats having elevated random blood glucose (RBG) concentrations far above 120 mg/dl were considered diabetic.

Reconstitution of the Ethanolic Extracts of Tithonia diversifolia Leaf

The crude ethanolic extract of *Tithonia diversifolia* leaves were reconstituted to obtain a concentration of 400 mg/ml by dissolving 1 g of the extract in 2.5 ml of distilled water, as descried for crude ethanolic extracts of *Anogeissus leiocarpus* stem bark [9, 12, 13, 14, 15, 16] and these earlier studies [9, 12, 13, 14, 15, 16, 17, 18, 19 20, 21] comprehensively exposed and confirmed *Anogeissus leiocarpus* as a standard

antidiabetic agent as utilized in animal grouping in the present study.

Feeding of Experimental Rats

All the rats received commercial feeds (Superstarter chow) and water *ad. libitum*.

Blood Samples Collections and Measurement of RBG

Blood samples were collected through the ocular vein and RBG values were measured as described earlier [17, 18]; briefly, a drop of blood was placed directly onto an Accu chek ® test strip inserted into a portable glucometer (Accu chek ® Active, Roche, Roche Diabetes Care, Middle East, FZCO).

Assay of Free Serum Sialic Acids

Blood samples for the assay of free serum sialic acids were collected from three each, of diabetic rats leaf extract treated (DEE*Td*L) and control non-diabetic rats (ND).

Free serum sialic acids concentrations were assayed using the Aminoff's principle [22] by applying the Quantichrome™ sialic acid Assay Kit (Bioassay Systems, 3191 Corporate Place, Hayward, CA 94545, USA) as utilized earlier [13].

Administration of Extracts, Withdrawals and Post-Withdrawal Monitoring

Oral administrations of the standard and the crude ethanolic *T. diversifolia* leaf extracts (under investigation) commenced immediately any of the rats developed hyperglycaemia in groups 3 and 4 respectively. When hyperglycaemia subsided in the rats of groups 3 and 4, oral administrations of the standard and the crude ethanolic *T. diversifolia* leaf extracts were withdrawn. Immediately thereafter, daily monitoring of RBG for any resulting normoglycaemia for fourteen days post withdrawal of standard and the crude ethanolic *T. diversifolia* leaf extracts and thirty-two days post induction of diabetes mellitus.

Body Weights

Body weights of the rats were measured at intervals during the course of the study.

Statistical Analysis

Students *t* statistical analysis was applied for the differences in the values of free serum sialic acids and p-value less than 0.05 was considered significant.

RESULTS

Phytochemical Screening of the crude Ethanolic Extracts of T. diversifolia Leaves

Details of the outcome from the phytochemical screening of the crude ethanolic extracts of *Tithonia diversifolia* leaves are presented in Table 1.

Alkaloids, Glycosides, Phenolic compounds, Tannins, Steroids, Carbohydrates, Flavonoids and Terpenoids were the phyto-constitutents detected in the crude ethanolic extracts of *T. diversifolia* leaves, while Saponins and Anthraquinones were absent.

Table 1. Phytoconstitutents of Crude Ethanolic Extracts of Tithonia diversifolia Leaves

Serial Number	Phytoconstituents	Present/Absent
1.	Alkaloids	+
2.	Glycosides	+
3.	Phenolic Compounds	+
4.	Tannins	+
5.	Steroids	+
6.	Carbohydrates	+
7.	Flavonoids	+
8.	Terpenoids	+
9.	Saponins	_
10.	Anthraquinones	_

Key: + = Present; **-** = Absent

Effects of Oral Administrations of Crude Ethanolic Extracts of Tithonia diversifolia leaves and Crude Ethanolic Extracts of Anogeissus leiocarpus Stem Bark (as standard) on Random Blood Glucose (RBG) Values in Alloxan-Induced Diabetic Wistar Rats

The variations in the values of RBG of the diabetic Wistar rats and the responses to oral administrations of the crude ethanolic extracts of *Tithonia diversifolia* leaves and *Anogeissus leiocarpus* stem bark are presented in Table 2.

RBG values in normal control, ND (group 1) ranged between 92 and 129 mg/dl during the study; diabetic untreated rats, negative control. DU (group 2) and diabetic rats, the orally administered the test sample, crude ethanolic extracts of *T. diversifolia* leaves, DEE*Td*L, (group 4) developed an overwhelming hyperglycaemia one day post induction of diabetes, except one rat in the latter group 4, which developed hyperglycaemia three days post induction of diabetes. Group 3 diabetic rats assigned for treatment with the standard, crude ethanolic extracts of *A. leiocarpus* stem bark, DEE*Al*SB, (group 3) developed hyperglycaemia eight days post induction of diabetes.

Diabetic untreated rats died from an overwhelming hyperglycaemia, by day 4 post induction of diabetes.

Oral administration of the standard, revealed its effectiveness and efficacy in treating diabetes mellitus as from four, five days, as from eight days post induction, and completely effective on seven, nine and eleven days post treatment of diabetes, which led to the withdrawal of standard on eleven days post administration.

The diabetic rats administered the test sample, crude ethanolic extracts of *T. diversifolia* leaves one day post

induction of diabetes, responded very slowly, displaying some levels of effectiveness five, six, twelve, fourteen and sixteen days post treatment with reversals in between, which led to its withdrawal eighteen days post administration.

Table 2. Effect of Oral Administrations of Crude Ethanolic Extracts of Tithonia diversifolia Leaves and Anogeissus leiocarpus Stem Bark on RBG (mg/dl) in Alloxan-Induced Diabetic Wistar Rats

Days post induction of DM	ND	DU	DEE*Al*SB	DEE*Td*L
1.	110, 116, 103	109, 112, 114, 116	112, 115, 116, 110, 114, 109,	118, 113, 120, 117, 114
2.	116, 129, 118	540, 580, 600, 480	100, 110, 106, 113, 108, 113	**Rx, 560, 112, 600, 600, 400
3.	120, 92, 98	560, 600, 600, 520	112, 108, 114, 116, 110, 115	597, 112, -, 308, 307
4.	127, 129, 126	590, 580, 560, 560	108, 114, 112, 115, 118, 119	-, 172, -542, 357
5.			110, 116, 114, 115, 108, 112	- - - 461, 176
6.			106, 114, 116, 112, 110, 108	**- - - 495, 122
7.			110, 112, 114, 106, 119, 115	**- - - 270, 102
8.			107, 112, 110, 103, 115, 119	- - - 507, 171
9.			**Rx, 139, 180, 68	- - - 436, 163
10.			117, 145, 560	- - - 526, 194
11.			124, 156, 560	- - - 512, 248
12.			116, 110, 563	- - - 528, 260
13.			111, 104, 428	- - - 388, 124
14.			117, 140, 600	- - - 513, 210
15.			** 111, 107	- - - 110, 136
16.			129, 140	- - - - 196
17.			** 100, 119	- - - - 100
18.			126, 149	- - - - 171
19.			**Wdxx** 107, 116	**Wdxx**- - - - 161
20.			107, 107	- - - - 127
21.			107, 134	- - - - 131
22.			123, 126	- - - - 231
23.			122	- - - - 185
24.			126	- - - - 181
25.			111	- - - - 209
26.			109	- - - - 179
27.			108	- - - - 195
28.			123	- - - - 127
29.			141	- - - - 219
30.			125	- - - - 175
31.			125	- - - - 141
32.			126	- - - - 195
33.			120	- - - - 170
34.			94	134

Key: **Rx = Treatment of (oral administration); **Wdxx** = Withdrawal of oral administration.

Some of the rats responded to the oral administrations so effectively, from an overwhelming hyperglycaemia to normoglycaemia over 24 hr and died of apparent hypoglycaemic shock

Effect of Alloxan-induced Diabetes Mellitus on Free Serum Sialic Acids Concentrations

The free serum sialic acids concentrations from three non-diabetic rats (ND) and three diabetic rats, leaf extract treated (DEE*Td*L) at the period of induced hyperglycaemia are presented in table 3.

The free serum sialic acids concentrations in the ND ranged between 0.9 mmol/L and 1.4 mmol/L while those in the DEE*Td*L group were elevated and ranged between 4.7 mmol/L and 5.6 mmol/L with mean values of 1.13 ± 0.15 and 5.23 ± 0.27 respectively. The difference was highly significant (P<0.001).

Table 3. Free Serum Sialic Acids Concentrations (mmol/L) of control Group of Rats (ND) and Diabetic Leaf Extract Treated (DEETlL) Group of Rats, at Period of DM

Serial No.	Control (n = 3) (ND)		Diabetic Rats (n = 3) (DEE*Td*L)	
1.	CR 1	1.4	DR1	5.6
2.	CR 2	0.9	DR2	4.7
3.	CR 3	1.1	DR3	5.4
Mean ± SD	**1.13±0.15[a]**		**5.23±0.27[b]**	

Key: CR = Control rat; DR = Diabetic rat

Leaf extract treated at period of DM

ab (P<0.001)

Oral Administrations of Crude Ethanolic Extracts of Tithonia diversifolia Leaves, Anogeissus leiocarpus Stem Bark and Body Weight (g) in Alloxan-Induced Diabetic Wistar Rats

Observation revealed that preponderance of heavily weighted rats were in group 3. Group 4 rat increased in weight by 31 days.

Table 4. Oral Administrations of Crude Ethanolic Extracts of Tithonia diversifolia Leaves, Anogeissus leiocarpus Stem Bark and Body Weight (g) in Alloxan-Induced Diabetic Wistar Rats

Days post induction of DM	ND	DU	DEE*Al*SB	Leaf DEE*Td*L
-1	101, 115, 111	105, 102, 104, 100	141, 127, 143, 143, 143, 120	126, 116, 125, 126, 129
+ 0				
+ 7			158, 130, 159, 148, 150, 123	107, 128
+14			162, 124	91, 129
+ 24			133	134
+ 31			143	140

DISCUSSION

Hyperglycaemia associated with the alloxan monohydrate – induced type 1 diabetes mellitus occurred in rats of groups 2 (DU), 4 (DEE*Td*L) one day post induction of diabetes mellitus and rats of group 3 (DEE*Al*SB) eight days post-induction of DM. Minor variations in the responses of the individual rats to the induction of diabetes mellitus was observed. Hence the display of the individual RBG values on Table 2.

The highly significant (P<0.001) elevation of serum sialic acids concentrations in rats of group 4 (DEE*Td*L) against the rats of group 1, (ND), the non-diabetic controls on day two post induction of diabetes mellitus is confirmatory of hyperglycaemia – induced endothelial injuries and the accompanying desialylation of endothelial cells of capillaries of body organs, other blood vessels and red blood cells [13]. Indeed, elevated serum sialic acids concentrations was established as a biomarker of diabetes mellitus in dogs [13] and elevated serum sialic acids predicted diabetes mellitus in human patients [23, 24, 25]; these led to the recommendation for laboratory assay of serum sialic acids concentrations of presenting obese human and animal patients [13, 21] for early diagnosis of prediabetes and to avail such patients the therapeutic potential of biomarkers in type 1 diabetes mellitus [26]. The highly significant elevation of free serum sialic acids reflects marked endothelial injuries and desialytion associated with the marked hyperglycaemia observed in the rats of groups 2 and 4 respectively, in the current study.

The oral administration of ethanolic extracts of *T. diversifolia* leaves to the diabetic rats ameliorated the hyperglycaemia, albeit, very slowly, in an erratic pattern and in one diabetic rat, 5, 6, 12, 14 and 16 days on administration, with very moderate hyperglycaemia re-occurring in between. The absence of a "fast" amelioration of the hyperglycaemia led to the death of some of the diabetic rats (see group 4; Table 2).

Upon withdrawal of the oral administration of crude ethanolic extracts of *Tithonia diversifolia* leaves and sixteen days thereafter, moderate hyperglycaemia persisted in the Wistar rat under investigation of the effect of withdrawal of oral administration. This suggests a continuous daily application of *Tithonia diversifolia* leaf for the control of diabetes mellitus; the favourable RBG values (127 mg/dl)

observed on days 2 and 10 post withdrawal is noteworthy (Table 2).

In group 3 (DEE*Al*SB) diabetic Wistar rats assigned as standard, conversely, induction of hyperglycaemia by alloxan monohydrate, was delayed, occurring eight days post induction, as against the group 4 (DEE*Td*L) which developed hyperglycaemia one day post induction.

A thorough scrutiny of all available results "revealed" a preponderance of very weighty Wistar rats in group 3, although rats were grouped at random. It is being suggested that heavier weights and hence large body mass index (BMI) of the rats in group 3 may have influenced and hence accounted for the delay of the induction of hyperglycaemia in this group 3. It must be emphasied that the large BMI is not related to or synonymous with obesity. Therefore, oral administration of the standard commenced eight days post induction due to the occurrence of hyperglycaemia, although one rat was hypoglycaemic and left untreated, on day 8. The standard ameliorated the hyperglycaemia immediately, but variously in the diabetic rats of group 3, upto ten days post administration of the standard.

Following withdrawal of oral administration of the standard, ten days post treatment and eighteen days post induction of hyperglycaemia, apparent normoglycaemia was maintained upto 16 days post withdrawal and thirty-three days post induction of hyperglycaemia, except for an inexplicable surge on day 28, eleven days post withdrawal.

This non-return of hyperglycaemic state, thus, the maintenance of normoglycaemia, after the withdrawal of the oral administration of the standard (group 3) very well supports and confirms an earlier finding that retention of normoglycaemia occurred after the amelioration of the hyperglycaemia of alloxan – induced type 1 diabetes mellitus in dogs administered crude ethanolic extracts of *Anogeissus leiocarpus* stem bark and the latter's withdrawal [14] suggested that treatment with *A. leiocarpus* prevented a progression to type 2 diabetes mellitus [14]. In addition, the antioxidant effect on diabetic Wistar rats [12], the prognostic effect signalling an ameliation of type 1 diabetes mellitus in dogs [13, 14] the attenuation of dyslipidaemia associated with alloxan – induced type 1 diabetic dogs [15] and the wound – healing effect in alloxan-induced diabetic dogs [16] justified the application of the crude ethanolic extracts of *A. leiocarpus* stem bark as standard in the current study.

Additional justification for the application of the crude ethanolic extracts of *Anogeissus leiocarpus* stem bark as standard, in the current study is derived from the successful and comprehensive purification, the wide range of safely value, lack of teratogenic effect in Wistar rats [17, 18] and the identifications of Lupeol [19] and Betulinic and Trimethoxyellagic acids [20] ascribed the responsibilities for the ameliorations of organic complications [14, 19] and hyperglycaemia [20], respectively characteristic of type 2 diabetes mellitus, as detailed elsewhere [21].

The apparent reduction in efficacy, in the amelioration of hyperglycaemia in diabetic rats by crude ethanolic extracts of *T. diversifolia* leaves and the subsequent non return to normoglycaemia after its withdrawal, when compared to crude ethanolic extracts of *A. leiocarpus* stem bark (standard) is noteworthy. A cursory examination of the outcome from phytochemical screenings revealed the absence of saponins and anthraquinones phytoconstuents in crude ethanolic extracts of *T. diversifolia* leaves, in the present study, while only anthraquinones was absent in crude ethanolic extracts of *A. leiocarpus* stem bark [17]. This observation may have some relevance from the antidiabetic properties ascribed to phytoconstituents/phytochemicals of medicinal plants [27, 28, 29, 30].

Studies showed that short term use of extracts of *T. diversifolia*, at lower doses, were effective and well tolerated in animals [8]. The toxic and therapeutic effects were ascribed to the bioactive principles present in *T. diversifolia*, namely, sesquitherpene lactones, chlorogenic acid and flavonoids with favourable safety index [8].

The phytochemicals, anthraquinones and saponins, were absent in the ethanolic extracts of *T. diversifolia* leaves. Anthraquinones, equally, absent in ethanolic extracts of *A. leiocarpus* stem bark [17], through Google search, National Institute of Health, Research Direct, Research Gate and Library search for peer review articles, [31] were described (with other names, anthracenedione or dioxoanthracene) as aromatic organic compounds with antibacterial, antioxidant, antiinflmmatory, laxative and diuretics medicinal activities; whereas, saponins present in *A. leiocarpus*, decreased blood lipids and may be associated with attenuation of dyslipidaemia of diabetic dogs [15] and lower blood glucose response; it is, in addition antibacterial and anti-inflammatory. It is being suggested that these saponins and Lupeol [19] present in *A. leiocarpus* stem bark contributed to the enhanced efficacy of the latter over *T. diversifolia* leaves in the amelioration of alloxan – induced hyperglycaemia in Wistar rats in the current study. However, the fractionation study, in progress, with *T. diversifolia* leaves shall provide more insight into the differences in efficacy.

The difference in the portion of the plant with the antidiabetic activity, leaves, of *T. diversifolia* as against stem bark in *A. leiocarpus* that served as standard may not account for the difference in efficacy observed in the current study. This opinion is from the backdrop that guill and perr leaf of *A. leiocarpus* were effective on the hyperglycaemia and associated dyslipidaemia in alloxan – induced diabetic rats [32] in addition, to the antihyperglycaemic activity of total extracts and fractions of *A. leiocarpus* in alloxan – induced diabetic mice [33] just as stem bark of *A. leiocarpus* effectively ameliorated hyperglycaemia, hepato-renal damages and

deranged electrolytes [14] and attenuated dyslipidaemia [15] in alloxan – induced diabetic dogs. However, the stem bark of *T. diversifolia* may not be readily amenable for favourable harvesting purpose, due to its lack of massive stem (see figs. 1 and 2).

The role of the phytochemicals of *T. diversifolia* in the amelioration of hyperglycaemia for the attainment of health in humans and animals, is a repetition of itself in ensiling processes of agriculture, for the improvement of silage for ruminants [7] by the removal of excess glucose from silage end-products. This speculation is derived from the relatively low blood glucose characteristic of older ruminants, where carbohydrate, hence energy metabolism, had been related to utilisation of volatile fatty acids, end-products of rumen microorganisms' fermentation of indigestible and digestible matter, such as propionate, lactic and butyric acids, although transient hyperglycaemia had occurred in cows [34].

CONCLUSION

Ethanolic extract of *Tithonia diversifolia* leaves has antidiabetic activity in alloxan-induced diabetes mellitus in Wistar rats. The amelioration of the overwhelming hyperglycaemia from the alloxan-induced diabetes mellitus in the Wistar rats, at the applied dose of 1000 mg/kg.bd.wt. was slow and delayed. Withdrawal of oral administration of the ethanolic extracts of *Tithonia diversifolia* leaves after amelioration of the hyperglycaemia produced a return to moderate hyperglycaemia, suggestive of a continuous daily oral use of *T. diversifolia* leaves as antidiabetic agent. Fractionation studies will provide an insight into how to enhance the efficacy of *T. diversifolia* leaf as an antidiabetic agent.

Ethanolic extracts of *Anogeissus leiocarpus* stem bark justified its use as a standard in this study. Body Mass Index appears to influence the "speed" of development of hyperglycaemia by alloxan-monohydate in Wistar rats.

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